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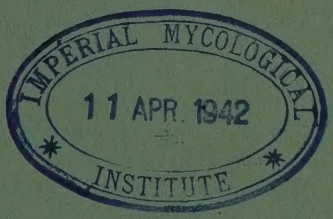

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VOL. I MAY, 1935 No. 5

REPRODUCTION AND LIFE HISTORY IN DIATOMS

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(Translated from the German by the editors)

Diatoms (Bacillariales) are unicellular algae with brown chromatophores. They are distinguished from all other algae by their cell-structure, mode of division and life history. The cell-wall is composed of two halves which fit one in the other like the top and bottom of a pill-box. Each half, in turn, is composed of two parts, the *valve,* which corresponds to the top or the bottom of the pill-box, and the *girdle band,* which corresponds to the side-wall. The valves are constructed radially, as in the Centrales, or they possess zygomorphic structure, as in the Pennales. During cell division the two halves separate from one another and each daughter cell produces a new half which fits inside the old one as the pill-box does within its cover. Since the valves are silicified and therefore neither extensible nor capable of further growth, the descendants which arise in each successive division are smaller and smaller.[1] This progressive diminution of the cells is counteracted, however, by occasional formation of particular cells, known as auxospores, which are capable of further growth. The essential nature of their formation is that a cell discards its rigid silicified membrane, thus liberating the protoplast which then grows to the size characteristic of the particular species. After this growth the auxospore again forms two silicified half-membranes. In many cases—and these are probably phylogenetically primitive ones—formation of auxospores is associated with a sexual act, insofar as the cell which bursts the membrane forms gametes or gamete-nuclei which fuse and produce a zygote; the zygote then begins to grow and becomes an auxospore.

In the Centrales there are, in addition, so-called microspores. These are small ciliated (possibly bi-ciliated) motile cells which are

[1] The general morphology of the diatoms has been summarized by Hustedt and by G. M. Smith (12, 21).

produced in numbers within the mother cell and then extruded. Their further history is unknown and it is uncertain, consequently, whether they are zoospores or gametes. The Pennales, on the other hand, do not have ciliated cells.

During recent years the propagation and life history of the Diatoms have been considered in several morphological, cytological and experimental researches. The alternation of haploid and diploid nuclei, which occurs among diatoms as well as in all other sexually reproducing organisms, was investigated at the same time. Since the Pennales and Centrales do not behave exactly alike, they may be separately considered in the following discussion, the Pennales first because they have been the more thoroughly studied. The general conceptions of cell diminution among the Pennales hold equally well for the Centrales.

PENNALES

Auxospores of the Pennales arise almost always from zygotes, that is, as the result of a sexual act. Vegetative development of auxospores represents loss of sexuality involving parthenogenesis or apogamy. Microspores and ciliated cells in general do not exist in this group.

The problem of cell diminution and, in connection with it, of the inducement of sexual reproduction or auxospore formation has recently been investigated, involving pure cultures and continued observations in nature on thirty species and varieties (9). Three categories of cells may be distinguished in each species. The first includes *cells of maximum size* which are capable only of cell division and of producing smaller progeny which in turn behave in a similar manner. A certain number of these divisions results in the second class, comprising *cells of much smaller size* which are capable of forming gametes as well as of cell division; these cells can produce auxospores. In species lacking sexual reproduction, auxospores arise from cells of this second size without preliminary gamete formation. Cells of the second class which, because of external conditions, do not produce auxospores, divide further and produce smaller and smaller progeny. These particularly small cells constitute the third category of *very small cells* which no longer are capable of reproducing sexually or of forming auxospores. They divide vegetatively under various depression phe-

nomena until finally their lowest vitality is reached and in all cases, even under the most favorable culture conditions, death ensues. The reason for this lies in the fact that, because of their smaller size, definite physical factors (relative surface, capillarity, surface tension) operate otherwise than in conformity with the organization of the species; not the least of such factors is the approach of the cell components to the molecular size.

Three principal phases of the life history may then be recognized: 1. the beginning of the life history with cells of maximum size; 2. the appearance of sexual or asexual auxospore formation in cells of smaller size; 3. the formation of still smaller cells which finally die and take no further part in the life cycle.

Only in cells of the second category, not in the larger or smaller ones of the first and third classes, can sexual reproduction or auxospore formation be secured experimentally. The essential requirement for its appearance is, under all circumstances, the cell size and correlated internal physiological conditions. In many species the necessary stimulus may be applied by transfer to a medium of weaker concentration involving change in turgor pressure. In like manner auxospore formation can be induced in *Melosira nummuloides,* one of the Centrales (20). In *Navicula seminulum* and *Gomphonema parvulum* light intensity also influences inception of auxospore formation, apparently through changes in turgor pressure resulting from formation of assimilates.

The effect of cell size, which determines physical-physiological changes within the cells, is generally more important than external conditions in deciding whether vegetative cell division or auxospore formation shall occur. Diatoms offer the most valuable material for the general problem of the relation between cell size and organization because they are favorably adapted to study.[2]

With decreasing cell size, there begin, during the course of cell divisions, characteristic morphological changes of shape and of valve-form. It is generally noted that the progeny of a cell is not

[2] One form, *Eunotia pectinalis* var. *minor,* shows an entirely different life history (9). Its cells possess girdle bands which are elastic and bulging in a characteristic manner, as a result of which the progeny do not necessarily become progressively smaller. In this species, also, auxospore formation was not observed. Incidentally it may be mentioned that some marine diatoms produce unsilicified membranes under certain cultural conditions (1, 2, 9). As the more or less abnormal cell-forms show, depression phenomena are involved in such cases.

merely a smaller geometrical likeness of its ancestor but that the cell occasionally possesses a definite shape which is coordinated with its size. It is most striking that one of the three cell axes (the apical or long axis) is shortened more than the other two and the cells thereby become more compact. These and other changes (reduction in bulging, decrease in the number of markings on the valve per unit of area) are of taxonomic importance. In considering material secured in nature, which includes cells of only one particular size, errors, such as the establishment of new species, arise because of failure to observe all changes in form. Changes associated with variations in size affect not only external morphology of the cells but also extend to the structure of the protoplast, involving, for example, changes in development of chromatophores and position of the nucleus; in short, simplifications are generally observed in connection with decreasing cell size.

In addition to shedding light upon form changes, the past years have brought increased cytological understanding, particularly with respect to changes in haploid-diploid phases of the nucleus. It has been shown, first, in confirmation of the classical investigations of Lauterborn, that the numerous newly investigated species, and this is true also for the Centrales, possess mitoses with a central spindle. The chromosomes, therefore, do not form a plate at metaphase but rather a ring (5, 6, 7, 15, 17, 18, 19). This type of mitosis, regarded as fundamental in animals, is very likely characteristic of all diatoms. A second result of recent investigations is that, in all sexually reproducing species, reduction division takes place during gamete formation; such species are accordingly diploid organisms. The Pennales, therefore, constitute the only group of plants which are definitely diplonts[3] like the Metazoa.[4] Therefore the old claims of Klebahn and Karsten, based upon only three species and partly incomplete, are confirmed upon a broad scale. Cytological details of meiosis have not been worked out, to be sure, in any case with the same clarity as in the Metazoa and Metaphyta. However, the fundamental similarity of meiotic processes which prevails in the

[3] For an excellent discussion of nuclear phases, alternation of generations and terminology among algae, especially the Red Algae, see Svedelius, Beih. Bot. Centr. **48**: 38–59. 1931.—Editor.

[4] In other *almost* diploid plants as the Fucaceae and the Angiosperms there is, on the other hand, a concealed antithetic alternation of generations, since a haploid generation (gametophyte), even though few-celled, does occur.—Author.

entire realm of living organisms can be established, likewise, among diatoms; that is, meiosis consists of two successive divisions, the heterotypic and the homotypic. Chromosome pairing takes place in the customary manner, progressing through the bouquet[5] stage to diakinesis[6] and finally to numerical reduction. The bouquet appears changed through fixation usually as synizesis.[7]

The best known and, because of their considerable size, the most favorable objects for studying these phenomena are *Cymbella lanceolata* and *C. cistula.*

The new investigations also make it possible to secure a complete picture of fusion processes (Cholnoky, Geitler). The gametes are, as has long been known, not ciliated but possess amoeboid movement. In one incompletely explained case (18) the existence of contractile vacuoles was established. These findings are of special interest since the diatoms, like all algae, are descended phylogenetically from the flagellates in which contractile vacuoles are characteristic.[8]

Fusion is initiated by juxtaposition of vegetative mother cells in which reduction division takes place and gametes are formed. Fusion of gametangia thus occurs. The mother cells of the gametes are often sister cells (paedogamy). Contact of the gamete mother cells is followed by bursting of the silica membrane and formation of a gelatinous envelope or fusion sac within which the gametes meet. The fusion sac is a transformation product of one layer of the membrane, composed of pectin and lying just within the silicified membrane in all vegetative cells (14).

The number of gametes is constant for each species and may be one or two. Pascher saw four naked protoplasts in cells of *Nitzschia* which were perhaps gametes; no fusion was observed, however. Since four cells or nuclei must have arisen almost in every case through the two divisions of meiosis, this condition in *Nitzschia* might easily be explained. In the other species two or three nuclei disappear and only one or two gametes develop. In

[5] The bouquet is that stage during meiosis in certain organisms in which the chromosomes lie in loops with their ends near one part of the wall of the nucleus (Darlington).—Editor.

[6] Diakinesis is the last stage in the prophase of meiosis immediately before the disappearance of the nuclear membrane (Darlington).—Editor.

[7] Synizesis is a contraction of the chromosomes to one side of the nucleus (an artifact) (Darlington).—Editor.

[8] Contractile vacuoles have been observed also in vegetative cells of *Rhizosolenia longiseta* and *Attheya Zachariasi* (13).

Cocconeis and *Navicula seminulum,* which form only a single gamete, one of the two daughter nuclei arising from the first (heterotypic) division disappears; the surviving nucleus undergoes the second meiotic division and one of the two daughter nuclei here too disappears; the remaining one develops into the gamete nucleus. While elsewhere supernumerary nuclei are resorbed into the protoplasm, in these species the first nucleus to disappear is abstricted from the cell, thus behaving like a polar body in animal oogenesis. This then represents a rudimentary second division. Formation of only one gamete must, therefore, be regarded as phylogenetic retrogression. Less rudimentary is the production of two gametes in one mother cell. The most primitive case, formation of four gametes, is to be found probably in *Nitzschia,* as noted above.

The gametes are entirely alike in both sexes or only slightly differentiated. In many species, however, they are distinctly different physiologically, since one gamete, the male, is motile and moves toward the other which is non-motile and female. There thus prevails a physiological anisogamy similar to that known in *Spirogyra.* If only one gamete forms in each mother cell, then the males penetrate those mother cells harboring the female gametes and there fuse with the latter. In the formation of two gametes, only one gamete of each cell is motile; the other is non-motile (resting gamete). The entire fusion process then occurs in two stages. A gamete of one cell enters the second cell and fuses with the gamete in it. At the same time, or a little later, the other gamete of the second cell travels in the opposite direction to the first cell and fuses with the gamete remaining there. In species which behave isogamously, the gametes meet halfway between the mother cells.

The physiologically anisogamous behavior involved in the formation of two gametes permits the interpretation that each mother cell forms two gametes of opposite sex, a male motile gamete and a female non-motile gamete. The mother cells themselves may, accordingly, be regarded as hermaphroditic and the entire behavior would, from this viewpoint, correspond to that involved in the conjugation of the Ciliates. So far as we know, sex determination occurs phenotypically in all cases hitherto investigated. A sex chromosome mechanism has not been observed nor can it be expected.

By means of fusion there arise one or two gametes from one

pair of mother cells, according to the number of gametes. Nuclear fusion follows in them. According to observations of Cholnoky (6) the sex nuclei during fusion show chromosomal structures of a prophase nature. The zygotes possess only one membrane which is burst during development of the zygote into an auxospore. Auxospore formation is, therefore, a germination process (Cholnoky, confirmed by Geitler, 1932).

Further growth of the auxospores, which is primarily a matter of expansion, ensues parallel to the direction of fusion in the case of isogamous fusion, whereas in the case of anisogamy it takes place perpendicular to the direction of fusion (Geitler). The two auxospores which arise from one pair of mother cells, therefore, are always parallel to one another but lie at right angles to the long axis of the mother cells in the first case and parallel to it in the second. These and other peculiar and constant position-relationships are dependent upon a definite polarity of the cells and need further investigation.

In the light of new and old investigations, the following review of the types of auxospore formation may be presented. Rare cases of retrogressive and lost sexuality are also considered.

1. Two mother cells each form two gametes which fuse in pairs and produce two zygotes (auxospores.)

a. The gametes are isogamous.

b. The gametes are anisogamous.

c. In some cases it can not be determined whether the gametes are isogamous or anisogamous, since the gelatinous sheath, within which fusion occurs, is very soft and the gametes can move about freely within it (9).

2. Two mother cells each form one gamete, resulting in one zygote (auxospore).

a. The gametes are isogamous.

b. The gametes are anisogamous.

3. In a single mother cell one zygote develops through automixis[9] (auxospore).

a. Two gametes develop in the mother cell which fuse and produce a zygote (auxospore).

b. Two surviving nuclei unite within the undivided protoplast to form a zygote nucleus; the protoplast then becomes a zygote.

[9] Automixis is self-fertilization following copulation of two closely related sexual cells or sexual nuclei (Gaumann and Dodge).—Editor.

4. Auxospore formation occurs without sexuality.

a. Parthenogetically; two mitotic divisions corresponding to meiosis occur which, however, are vegetative divisions (diploid parthenogenesis) as in metazoan eggs.

c. Apogamously; completely lacking all indication of sexuality.

Some special peculiarities may well be included in this review (9). In *Cymbella sumatrensis* vegetative nuclear division without cell division takes place in the auxospore which has arisen according to Type 1b. One of the two daughter nuclei disappears and the second becomes the true nucleus of the auxospore which then develops normally. An accurate interpretation of this process is not yet possible; it may be supposed, however, that a preliminary rudimentary cell-division does take place which is represented by the surviving nuclear division. In this connection it is of interest that the first division of the auxospore in species of *Cocconeis* proceeds irregularly and produces two unequal daughter cells. The one daughter cell has normal structure and continues to develop; the other is always irregular in a definite manner and has no raphe.[10] This second cell is not capable of further normal development. So in this case also only a single normal cell forms after a division.

In conclusion, it may be said that the Pennales possess primary sexual reproduction. Alternation of nuclear phases is apparent because meiosis enters into gamete formation. The Pennales, like the Metazoa, are, therefore, diploid organisms. Auxospore formation occurs simultaneously with zygote production. Many species have lost sexual reproduction and in them auxospores arise parthenogetically or apogamously.

CENTRALES

The general life history of the Centrales is, for the most part, the same as described for the Pennales. Here, also, the cells become smaller and smaller during the course of cell divisions and the original size is restored through formation of auxospores.

While in the Pennales the general principles of sexual reproduction and auxospore formation are understood, this is not true for the Centrales. During recent years several investigations have been directed at this problem but without yielding conclusive general

[10] The raphe is a cleft in the membrane through which streaming protoplasm protrudes, causing the well known creeping movements of the cells.

clarification. The present status of the investigations may now be discussed.

The most significant deficiency in our understanding is that the behavior of the microspores has never been exhaustively observed. The old assumption of Karsten, that the microspores fuse with each other isogamously, is unproven, since it is not supported by observation. Schmidt (19), working with *Biddulphia,* and Hofker (10) with *Coscinodiscus,* recently advocate the same idea, but without having observed fusion of the microspores. The opinion of these authors is founded on the fact that divisions take place during formation of microspores which might be regarded as constituting meiosis. The cytological picture, however, is not so clear as in the Pennales and the evidences of meiosis are not entirely conclusive. It is possible, nevertheless, that the interpretation of Schmidt and Hofker may be correct, in which case the microspores would be isogametes which fuse with one another and the Centrales which have been investigated would be diploid organisms like the Pennales. In contrast with the Pennales, however, formation of zygotes would not be associated with production of auxospores; the auxospores would develop vegetatively instead and at another point in the life history.

Persidsky (17) made entirely different observations in connection with *Chaetoceros.* At the beginning of auxospore formation the nucleus undergoes two divisions; of the four daughter nuclei two disintegrate while the other two fuse within the plasm of the mother cell and become the auxospore nucleus. Persidsky regards the two cell divisions as constituting meiosis. Autogamous fusion, therefore, takes place and the general behavior is the same as in Type 3b of the Pennales. Auxospores would then arise from zygotes and the behavior would agree fundamentally with that of the Pennales. Persidsky regards the microspores as zoospores. The cytological observations are certainly not sufficiently detailed that this idea may be regarded as definitely established in all respects. It is particularly significant that three of the four nuclei are smaller than the fourth, almost conveying the impression that three nuclei disintegrate rather than two. If three nuclei actually do disappear, the ensuing fertilization must be by a gamete from some other source. The following observations are particularly significant in this connection.

In another species of *Chaetoceros,* F. W. Went made some observations which, though incomplete and therefore inconclusive, may perhaps supply the key to an understanding of the entire problem of reproduction among the Centrales.[11] Went observed that cells with undivided contents were surrounded by microspores which had been formed in other cells. Went assumes that this was the initial stage of a fusion; the actual fusion, however, was not seen. If fusion does occur, then the microspores would be regarded as small male gametes (spermatozoids) which escape from the mother cells and swim to the female cell, which in turn contains an undivided female gamete (egg-cell). The contents of the undivided cells would, therefore, be fertilized and the resulting zygotes would develop into auxospores.

If these assumptions are correct, then the contradicting claims of Schmidt and Hofker on one side and of Persidsky on the other may be accounted for. If the microspores are spermatozoids, we can understand why no one has observed fusion among them, although there is meiosis during their division. Schmidt and Hofker may, therefore, have observed only the formation of male gametes; Persidsky, on the other hand, may have investigated female cells in which reduction division likewise occurs, followed by formation of zygotes (auxospores). Nuclear fusion, however, would not occur through autogamy, as Persidsky claims, but would be the fusion of the fourth surviving nucleus with one nucleus of a penetrating microspore. In any case, Persidsky did not observe microspores. It is quite possible, therefore, that his claim for autogamy is correct, all the more so because autogamy does occur in *Melosira.* Different species can behave in different ways, either as described by Went or by Persidsky. Likewise among the Pennales some species of one genus show autogamy (*Achnanthes subsessilis* according to Karsten), while others show allogamy (*A. lanceolata* according to Geitler).

If the above mentioned conception is correct, then a general understanding of all Centrales may be possible, though at present it may be only a working hypothesis. The Centrales appear to be phylogenetically the more primitive group in contrast with the Pennales, since the former exhibit fusion of gametes and the latter

[11] A short article appears in Nederl. Chron. Arch. 1924–25. A letter received by me on this subject is presented on page 11 of the 1932 volume.

fusion of gametangia. The fundamental uniformity of diatoms, which undoubtedly exists in their vegetative structure, is apparent also in their reproduction, since in all cases sexual reproduction and reduction division appear to be associated with auxospore formation.

Of great significance in this connection are the new investigations on *Melosira*. Cholnoky observed in *M. arenaria* that the cells which form auxospores contain three nuclei, one of which is larger than the other two. The two small nuclei disintegrate and the larger one becomes the nucleus of the auxospore. Cholnoky thinks that meiosis occurs before auxospore formation, that two of the four nuclei perish and that the two surviving ones fuse with each other. Autogamy would then prevail, as Persidsky claims, for *Chaetoceros*. Persidsky was able to prove this assumption to be an actual fact in *Melosira varians* without knowing the observations of Cholnoky. In mother cells which later develop into auxospores, he observed two nuclear divisions which represented heterotypic and homotypic divisions of meiosis. The cells, therefore, contain four nuclei, of which two degenerate while the other two fuse with each other and the syncaryon[12] becomes the nucleus of the auxospore. The latter, therefore, arises from an autogamously formed zygote.

It is accordingly certain that auxospores in *Melosira* do not arise purely vegetatively, as other authors have assumed to be a general condition in the Centrales, but that their formation is associated with a sexual act. Microspores are not known in *Melosira* and the problems concerning them, therefore, still remain unsolved. According to the working hypothesis stated above, *Melosira* may be regarded as phylogenetically retrogressive in contrast with other Centrales which possess microspores. In comparison with *Coscinodiscus* and *Biddulphia, Melosira* is more closely related to the Pennales. As far as reproduction is concerned, it behaves exactly like group 3b of the Pennales.

SUMMARY

The Pennales are diplonts and possess sexual, parthenogenetic or apogamous auxospore formation. Fusion is either isogamous or physiologically anisogamous.

[12] The syncaryon, here, is the fusion product of the two surviving nuclei.—Editor.

Sexual reproduction among the Centrales, with the exception of *Melosira,* is not fully understood. It is probable, however, that the Centrales are also diplonts. Contradicting claims of various authors can be explained through the assumption that the microspores are spermatozoids which fertilize the undivided contents of female cells (egg cells) and that the resulting zygotes develop into auxospores. Conclusive proof of this claim is lacking for neither do cytological investigations of reduction divisions which occur during microspore formation suffice in the matter, nor has fusion of the gametes been observed. In *Melosira,* which has no microspores, reduction division takes place in the mother cells before auxospore formation; two of the four nuclei fuse and the zygote develops into an auxospore.

The problems concerning sexual reproduction and alternation of nuclear phases among Centrales still remain unsolved except for *Melosira.* The Pennales, on the other hand, are definitely diploid.

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AUXIN, THE PLANT GROWTH-HORMONE

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I

Over fifty years ago Julius Sachs (48) clearly pointed out that correlations in plants most likely were caused by "specific substances," not of the nature of foodstuffs but directing the activities of cells in extremely small quantities. Twenty-five years later hormones in animals were discovered, substantiating the principal point of Sachs's theory; but it took another twenty-five years before botanists generally became aware of the soundness of his reasoning. It is clear now that plants furnish most favorable material to study "correlation carriers" or phyto-hormones. The following report on the plant growth-hormone or growth-substance (g. s.) substantiates this statement. The physiological name *growth-substance* and the chemical name *auxin* are interchangeable.

Of the different growth stages (initiation, differentiation, elongation and maturation) elongation is the most spectacular and the one that can best be measured since it involves the greatest change in dimensions. It is in this stage of cell elongation that the more or less embryonic cells of organ primordia enlarge to their final size. This elongation is, in most cases, accompanied by some cell divisions, which, however, are of minor importance. In *Avena* coleoptile,[1] which is the main experimental plant in investigations on g. s., no cell divisions occur in the stages in which it is used (61) and any factor affecting growth influences cell elongation only.

Now growth in length furnishes one of the finest examples of correlations in plants. For a long time it has been known that most stems, petioles, flowerstalks and coleoptiles stop growing if the apical, only slightly growing parts (end buds or leaf blades, flowers, or tips, respectively), are cut off. Paál (46) was the first to demonstrate that this is not due to a simple wound shock, for, if the tip of a decapitated coleoptile is replaced on the cut surface, the

[1] The coleoptile is a leaf-sheaf which envelopes the growing point and first foliage leaf.—Editor.

stump will grow faster than without the tip (Fig. 1: demonstration) (52). It appears that this influence of the tip is caused by some substance diffusing out of it, as a layer of gelatin will not impede this action. For many other plants and organs it can be shown (53, 73) that replacement of the cut off apical parts at least partially restores the original growth rate.

II

As a consequence of this and other work Went (75) demonstrated that the growth promoting factor from coleoptile tips will diffuse out of them into a layer of agar or gelatin if they have been standing on it for some time (Fig. 1: collection). A set of decapitated coleoptiles will grow considerably faster if blocks of this agar are placed on their cut surfaces than if they are supplied with pure agar (Fig. 1: demonstration). This principle of influencing growth with the exudate of tips, diffusing out of agar blocks, has been worked out as a quantitative method of determining the amount of growth-substance dissolved in agar (75, 78). To rule out more or less individual variations each determination of g. s. is made with 12 plants. They are raised under exactly constant conditions: darkness (only red or orange light is used to carry out the manipulations), constant temperature of 20° or 25° C., constant humidity of 90 per cent and absence of traces of toxic gases. When the plants have reached a length of 30–40 mm. they are decapitated and their first leaf (inside the coleoptile) is pulled loose to prevent it from growing out and pushing the agar block off the cut surface (Fig. 1: detection). An agar layer, 8 x 6 x 1 mm., containing auxin is cut into 12 equal pieces, 2 x 2 x 1 mm., so that each little block carries the same amount of g. s. (Fig. 1: collection). Forty minutes after decapitation such an agar block is stuck on one side of the cut surface of each of the 12 plants (Fig. 1: detection). As the transport of the hormone is strictly longitudinal, only the side under the agar block will have an extra supply of g. s. and, consequently, will grow faster than the opposite side. This results in a curvature away from the agar block, which is measured about 2 hours after application of the agar (Fig. 1: detection). Over a range of about 1° to 20°, curvatures are strictly proportional to concentration of g. s. in the agar (Fig. 1, lower right; 65). Thus, the mean curvature of 12 plants provides an accurate

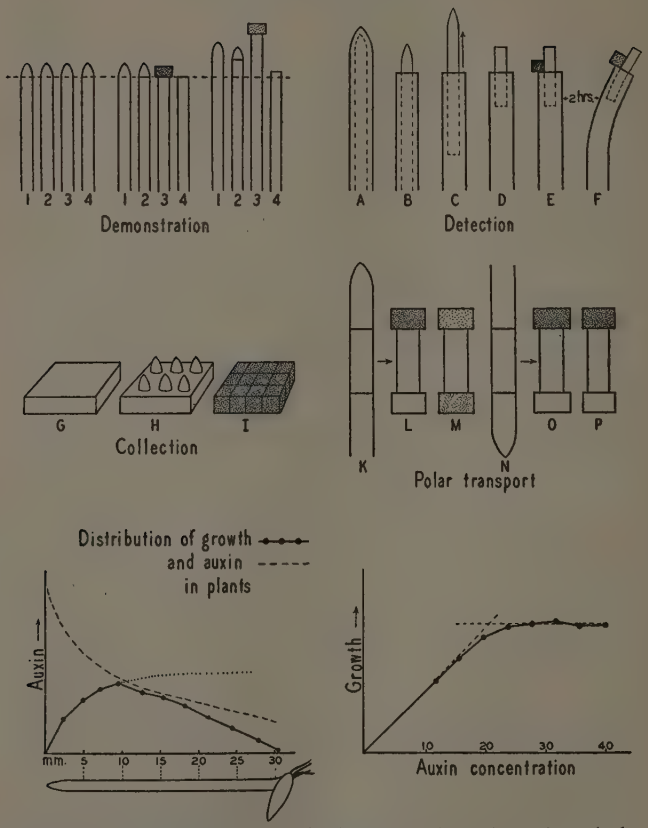

FIG. 1. Auxin (its concentration in the agar is shown by various shades of dotting) and growth of *Avena* coleoptiles.

Upper left. Demonstration of auxin formation by coleoptile tip. Plant No. 1 is left intact; Nos. 2, 3 and 4 are decapitated. On No. 2 the cut tip is replaced; on No. 3 auxin (in agar) is stuck. The right hand set of four plants shows the effect of this treatment on growth after three hours.

Middle left. Collection of auxin from cut coleoptile tips. For a period of 2 hours 6 coleoptile tips are placed (H) on a layer of agar, 6 x 8 x 1 mm. (G). After removal of the tips the agar contains auxin diffused out of the tips, and is cut into 12 blocks (I).

Upper right. Scheme or test method for auxin. Coleoptile (A) is decap-

determination of the concentration. If blocks of agar have been soaked for an hour in some solution, its g. s. content can be calculated, as auxin easily diffuses into the agar. This content is expressed in arbitrary g. s. units which are reproducible under exactly defined experimental conditions. Kögl and co-workers accept the A. E. (*Avena* Einheit) which is the amount of auxin present in one block of agar, 2 x 2 x .5 mm., causing a curvature of 10°. The auxin unit of the Kerckhoff Laboratories, Pasadena, expresses the amount of g. s. present in 1 cc. of solution and equals 80 A. E. A plant unit is the amount actually applied in agar and is 1/200 of this amount. The *d*-Werte of Nielsen (43) have not been compared directly with the above units. For qualitative work auxin can be mixed with lanolin (39) which prevents the desiccation inherent in working with agar blocks in a dry atmosphere. Recently a new and simpler test method for g. s. has been described (77) using pea seedlings as experimental plants which are placed directly in the solutions to be tested.

III

With the *Avena* test the chemistry of growth-substance has been worked out especially by Kögl, Haagen Smit and Erxleben (29,

itated leaving the primary leaflet (B). The latter is partly pulled out (C and D) and an agar block with auxin is placed on one side of the cut surface of the coleoptile (E). Two hours after application of the agar the resulting curvature (F) is measured.

Middle right. Demonstration of polar transport of auxin. On the apical surface of a coleoptile cylinder cut from the seedling (K) a block of agar with known auxin concentration is placed and the basal surface is placed on a block of pure agar (L). A few hours later the greater part of the auxin will have been transported towards the lower block (M). If the coleoptile cylinder has been reversed (N and O) no transport whatsoever is detectable; all auxin remains in the upper block, in contact with basal cut surface (P).

Lower left. On the abscissa zones of a coleoptile (in mm. from the tip) are marked; on the ordinate growth rate (corresponding with drawn line) and auxin concentration (corresponding with broken line). From 9 mm. downwards growth rate is determined by auxin concentration (with allowance for "aging" of zones more than 20 mm. from tip). In the upper 9 mm. a hypothetical factor (extended as dotted line) limits growth rate.

Lower right. Relation between applied auxin concentration (abscissa) and rate of curvature or growth (ordinate). Below a certain point (marked 20) growth rate is directly proportional to auxin concentration; above this point a second internal factor starts to limit curvature or growth rate.

35). Also Nielsen (43), Dolk and Thimann (17), and Thimann (63) contributed valuable work along this line. Kögl and coworkers have isolated three different crystalline substances, all giving positive reaction in the *Avena* and pea test, and, therefore, being growth-substances; physiologically they can not be distinguished. They have been named auxin *a* ($C_{18}H_{32}O_5$); auxin *b* ($C_{18}H_{30}O_4$);[2] and hetero-auxin (β-indolyl-acetic acid, $C_{10}H_9O_2N$); and all three of them are monobasic acids of about the same strength and have one double bond. Auxin *a* forms a lacton of the same empiric formula as auxin *b*, which is also active as growth-substance.

Auxin *a* and *b* are heat- and light-stable but are easily oxydized; the crystals lose their activity in a few months by isomerisation. They are readily oxydized by oxydase from the plant cells; this explains why ordinary water extracts of plant tissues do not have any effect upon growth (62). The activity of all three auxins is about the same and extremely high, of the order of 5×10^7 A. E. per mg. The *Avena* test thus is one of the most sensitive microchemical tests. No other pure substances have been found to have any direct effect on growth at all; if saliva, diastase or other products have a growth accelerating effect, this is due to traces of one of the auxins.

One of the richest sources for auxin preparation, human or mammalian urine, contains from 1000 to 5000 A. E. auxin per mg. From malt as well as from corn-germ-oil both auxin *a* and auxin *b* can be prepared (32). Micro-organisms produce auxin (apparently hetero-auxin (33, 63)) in large quantities, synthesizing it from the culture solution (43, 29, 67, 7). Pollen and seeds are the richest higher plant sources of auxins (38, 69).

IV

The three auxins are physiologically indistinguishable, all of them giving the same type of growth response. Because they are analyzed by physiological tests it is very difficult to say anything general about the distribution of auxin *a* and *b* and hetero-auxin in relation to the natural system of the plant kingdom. The crystals of auxin, of course, are recognisable by their melting point,

[2] Auxin *b* is in no way related to *B*-Wuchsstoff of Nielsen; it is, like the other auxins, an *A*-Wuchsstoff.

etc., but Kögl, *et al.* (32, 34) succeeded in distinguishing between them even in impure solutions by their different behavior toward acids and bases, and by their diffusion coefficient which allows the approximate calculation of the molecular weight (75).

Auxin *a*: stable in acid, destroyed by alkali; mol. weight 328.
Auxin *b*: destroyed by acid, destroyed by alkali; mol. weight 310.
Hetero-auxin: destroyed by acid, stable in alkali; mol. weight 175.

On account of these properties it was possible to show that in coleoptile tips of corn auxin *a* is formed. From corn-germ-oil as well as from malt crystalline auxin *a* and *b* can both be prepared. So it seems probable that in the higher plants auxin *a* (and perhaps auxin *b*) are formed as *the* growth-substance. In the lower plants Kögl and Kostermans (33) and Thimann (63) could prove that hetero-auxin is formed, and not auxin *a* or *b*, probably from the tryptophane of the culture medium. Rhizopin (43) must be identical with hetero-auxin.

About the distribution of auxin inside a plant almost nothing is known, owing to its inactivation in plant extracts. Only the regions of production are known: end buds, axillary buds (68), leaves (1), cotyledons and coleoptile tips (and perhaps root tips, see paragraph 8). Thimann (62), however, by direct extraction with chloroform could determine the actual concentration of auxin in the *Avena* seedling. It is highest in the tip where it is produced and thence drops towards the base where it is used up in growth.

In most plants the cutting of a g. s. production center means irreversible loss of the capacity of auxin formation. In coleoptiles of grasses, however, all cells potentially possess this capacity. Some hours after the tip has been cut off the cells nearest the cut surface will start to produce auxin again and growth of the stump is resumed. This regeneration of the physiological tip depends upon a number of circumstances such as temperature (70), place of decapitation (82), presence of a free cut surface (60). It is significant for the importance of auxin production in the plant that the sensitivity for stimuli is almost lost during the period between cutting of the tip and regeneration of the physiological tip, but is restored immediately afterwards (15). Perry (47) has described cytological changes in the cell connected with auxin production.

In some organs no center of g. s. production has been found but

it seems to be present everywhere; in such plants decapitation does not have any influence on growth rate. *Lupinus* hypocotyls belong to this type (14). However, if the central cylinder containing the vascular bundles has been cut out, the cortex tissues considerably decrease their growth rate which is restored by addition of growth-substance (12). In this case, the phloem seems to contain or produce the auxin. It is assumed that the formation of g. s. in coleoptile tips involves the transformation of a precursor—in itself inactive—into the physiologically active form. No direct evidence has been brought forward to support this view but many phenomena like regeneration can be explained by it. Another explanation of this regeneration (68) assumes that formation of auxin occurs in the lower zones of growing organs only if their auxin content falls below a certain value; the authors conclude no regeneration can take place there as long as formation in the tip goes on. After decapitation the g. s. concentration decreases resulting in the possibility of regeneration in the lower cells.

We are but poorly informed about the effect of light on auxin production. It drops in prolonged darkness; so, in the long run, light is necessary (45). Only in seedlings is g. s. formed in the dark, apparently from reserves in the seed. In *Avena* coleoptile it is also known that light has a direct effect on diffusion of g. s. from the tip into agar; this phenomenon explains the "long" light-growth reaction (75, 9). In *Raphanus* cotyledons no such effect was found (45).

V

Experimental work, especially by van der Wey (79, 81), on transport of auxin in the plant has produced results which are remarkable not only in themselves but also from a general standpoint. Auxin is the only substance, occurring naturally in the plant, whose translocation can be followed directly, as almost 100 per cent of the administered growth-substance can be recovered or accounted for by the *Avena* test. The experiments are carried out by placing two blocks of agar, one with and one without auxin, on either a cut surface of a coleoptile cylinder or other tissue to be investigated (Fig. 1: polar transport). If the original concentration of g. s. in the agar is known and the final one (*e.g.*, after an hour's transport) is determined experi-

mentally, the transport can be calculated. In the first place it is found that rate of transport exceeds rate of diffusion by many times. Now we must distinguish between velocity and capacity of transport. The velocity, about 10 mm. per hour, is hardly influenced by temperature and not at all by the length of the tissue through which it passes, nor by the concentration gradient. The capacity of the transport (the amount of auxin transported per unit of time) depends on the concentration gradient but not on the length of the path of transport; whether g. s. has to be transported through a 2 or 6 mm. cylinder does not change the amount transmitted. The effect of temperature on capacity (in contrast with velocity) of transport is very marked; under 40° it is a typical optimal curve with a sharp optimum at 30°–35°; above 40° a completely unexplained second optimum occurs at about 50° All these facts (except the last) point to the conclusion that transport of g. s. inside the plant is intimately connected with life processes and is not explainable by physical forces alone. In agreement with this conclusion is the fact that narcotization with ether stops transport beyond diffusion rate completely. This inhibition is reversible within certain limits of ether concentration. In the lower ones only the capacity of transport is decreased, the velocity remaining normal (81).

Bottelier (6) recently investigated protoplasmic streaming in *Avena* coleoptile in its dependance on temperature, light, gravity, etc. The velocity of streaming (about 30 mm. per hour) is about constant between 17° and 35°, at least in young coleoptiles which are used for g. s. experiments. Intensity of streaming (*i.e.,* the amount of protoplasm in actual rotation), however, increases with temperature up to an optimum at about 30°.

There is a close parallelism between auxin transport and protoplasmic streaming, and as transport is too fast to be explained by diffusion, the old theory of de Vries seems to explain the facts: namely, auxin is transported inside the cell by protoplasmic streaming which is about 3–4 times as fast as the observed auxin transport velocity. Resistance in transport has to be sought, then, in diffusion of g. s. from one cell to the next, for it has been found that in the coleoptile transport of g. s. takes place through long parenchyma cells and not primarily through the vascular bundle.

A second group of phenomena is connected with direction of g. s.

transport. Auxin is transported only basipetally, *i.e.*, a transport against polarity towards the tip does not take place (75, 79, 81, 45, 37). This polarity also reversibly disappears upon narcotization (81). A still more remarkable demonstration of polarity in growth-substance transport lies in the fact that it is transported against a gradient: an auxin concentration at the base of a coleoptile cylinder of 10–20 times the concentration at the apical end does not impede transport in a basal direction (79). To cover these facts the theory has been proposed that naturally occurring potential difference in the coleoptile and other organs (apical parts being negative against basal parts) would produce an electrophoresis of the negatively charged ions of auxin towards the positive base (76, 28). This is, however, not the place to discuss this theory; it is only mentioned because tropistic curvatures are generally explained now on an electrical basis.

VI

The properties, formation and transport of auxin having been described, using the *Avena* test as a means of investigation and not as a problem in itself, we have next to consider what part g. s. plays in causing curvature, *i.e.*, in growth. It has been found, at least in *Avena* coleoptiles, that auxin is an indispensable prerequisite for growth in length; without growth-substance there is no growth (75, 16). If an *Avena* coleoptile is decapitated growth rate drops steadily (because the auxin still present in the tissues after decapitation is gradually used up), until regeneration of the physiological tip. If the latter is cut off just before it starts to form g. s., regeneration is delayed for another few hours and growth mostly completely stops. During this period of arrest growth can be restored immediately at any moment by application of auxin. In this case growth is directly proportional to the amount of auxin applied and even can be increased above normal. But soon a limit is reached above which increase of applied auxin concentration does not increase growth any further (Fig. 1: right hand graph) (66, 14). This means that g.s. is no longer the factor limiting growth but one of the other factors, on which the complicated process of growth depends, begins to limit it; from that moment on, auxin is present in excess. This certainly is one of the finest examples of Blackman's theory of limiting factors for there is only a very short

zone of transition where auxin together with the other factor influences growth rate. These facts together with results of decapitation experiments enable us to draw the conclusion that in the normal plant it must be mainly auxin which determines growth rate; only if growth is increased beyond normal do other factors become limiting. (Demonstration of this is furnished by the left hand graph on Fig. 1, where growth rate of a coleoptile appears to be determined, at least in the lower zones, by its auxin content.) The same limitation is met in experiments with unilateral application of g. s.; over a certain range curvatures are directly proportional to concentration but above that they can not be increased (Fig. 1: right hand graph). Then some other factor limits rate of curvature (75, 43, 78) and it is likely that this is not the same factor as in straight growth experiments (10).

Curiously enough our knowledge of this second growth factor is restricted to suppositions. It may be that it is a second hormone, transported upward from the seed or roots, or it may be identical with "cell stretching materials" (75) or perhaps "aging" of the cells has something to do with it (9). As "aging" we have to understand the fact that old cells hardly react at all with auxin. By experimentally stopping growth for some time (by decapitation) cells will be aged and their growth rate will irreversibly be decreased. That lower zones of a young organ gradually stop growth must be explained, then, not only by decrease in auxin concentration (75) which falls towards the base, but also by decreased reactivity of the "aged" cells further from the tip (Fig. 1: left hand graph 1). Still, in some cases at least, growth may stop because all g. s. has been used up in more apical parts. In those cases, growth will be resumed (though slowly, because the cells "aged" during the arrest of growth) upon auxin application (75).

VII

The question of the mechanism of auxin action has given rise to a considerable number of papers by Heyn, Söding, Bonner, and Strugger. The main result of their investigations is that auxin reacts with the protoplasm of a cell only as the last link in the reaction chain: g. s.—growth, the cell wall properties are changed, causing their elongation. Intermediate stages are still completely unknown.

Growth in length of a cell might be induced either by increase of the force stretching the cell wall, that means by osmotic pressure, or by change in mechanical properties of the cell wall. From previous work, especially by Ursprung and Blum (72), it is clear that osmotic pressure of the cell sap does not increase in growing cells; on the contrary, it may slightly decrease, whereas suction force increases. From this fact the conclusion can be drawn that in growing cells, in the first place, extensibility of the wall changes. Though turgor is a formal necessity for growth it is not its cause.

It has been possible to show (23, 26, 27, 54) that it is primarily plasticity of the cell wall which changes during growth. Direct proof has been given that this plasticity is increased by auxin. This means that the cell wall can be stretched irreversibly by turgor. At the same time elastic extensibility may or may not increase; these changes in elasticity are correlated with growth but not with g. s. Increased elasticity is a result of growth, increased plasticity its cause. That these two properties of the cell wall change independently (another instance is that at 0° C. no changes in elasticity occur, whereas plasticity increases upon g. s. supply) has been explained by a lamellar structure of the cell wall (24). An investigation has been started by means of X-rays to study the effect of auxin on the molecular structure of the cell wall (25).

Lately, Söding (56) has assumed that the primary cause of growth lies in the active intussusception of new material in the cell wall. However, at 0° C. growth caused by auxin takes place normally whereas intussusception is stopped (5). Also from other experiments it is clear that intussusception and growth are two independent phenomena. If the latter is stopped by lack of auxin, the former may go on, making the cell wall more rigid and less stretchable. In other words, this is "aging" of the cells (9). In normally growing cells increase in substance, *i.e.*, in rigidity, by intussusception is kept in balance by plastic stretching. We can conclude these considerations with the paradox that growth of the cell wall, as measured by increase in solid matter, is opposed to the actual growth process we are studying, namely, growth in length by auxin. In this respect an interesting article on growth of the seta of *Pellia* (hepatic) of Overbeck (44) may be mentioned. It brings a number of new observations relating to the problem of growth as passive stretching only or as active intussusception.

Bonner has investigated stages of auxin action preceding change in plasticity. Aerobic metabolism must take place to induce the latter for growth can be inhibited by the same concentrations of KCN and phenyl-urethane which inhibit respiration. Also in a nitrogen atmosphere no growth takes place. On the other hand, there is no appreciable increase in respiration upon application of auxin (the effect of crude g. s. preparations on respiration is due to impurities), so that the part of respiration necessary for g. s. action must be extremely small. The conclusion is reached that respiration is a formal prerequisite for growth; without respiration there is no auxin action.

In several papers Strugger (57–59) demonstrated that growth can be induced also by application of acids. The acids were supposed to act directly on the protoplasm, shortcutting the stages of auxin action by increasing its imbibition pressure in general, resulting in increased growth. At the iso-electric[3] point of the protoplasm (pH = 5.1) hydration would be lowest and growth would fall to a minimum. In this general theory of growth many facts are disregarded, especially the very specific action of auxin on growth. Bonner (4) was able to show, at least in *Avena* coleoptile, that the effect of acid on growth has nothing to do with physical properties of protoplasm but can be explained quantitatively by the setting free of a certain amount of undissociated auxenolic acid, the chemical form in which auxin is active. In contrast to Strugger he did not find a double growth optimum in different acidities. The simple optimum in acid surroundings is caused by change in acidity of cell contents produced by application of acid buffers; this activates most of the auxin still present in the cell at once. No growth stimulation by acid beyond the amount corresponding with the auxin originally present in the cell has been observed. The figures of Gessner (20) on plasticity of cell walls in different pH[4] suggest that the same explanation holds for *Helianthus,* Strugger's object.

VIII

In the preceding discussion roots have not been mentioned because of their exceptional behavior to auxin. Their growth is

[3] The iso-electric point is that degree of acidity or alkalinity of a medium at which a substance behaves neither as a base nor an acid.—Editor.

[4] pH values indicate degrees of acidity and alkalinity; 7 is neutral, higher values are alkaline, lower values acid.—Editor.

almost completely limited to the extreme tip. Growth in length is a mixture of cell division and cell elongation which are going on at the same time.

Also, in roots, decapitation has an effect on growth. If only the extreme tip has been cut off, a correlation effect causes an increase of growth rate (12). As in the coleoptile, replacement of the tip more or less restores normal growth; this means it decreases growth rate. It was obvious to suppose that here also auxin was producing the effect, especially as the coleoptile tip exerted the same influence as the root tip. Indeed, Nielsen (43) and Boysen Jensen (8) could show that growth of roots stops completely if they are placed in an auxin solution. The same experiment was repeated by Kögl (34) with crystalline auxin with the same result. If applied to one side of the growing zone of the root, curvatures towards the g. s. appear, also indicating a decrease in growth rate (39).

For some time the source of g. s. in roots was unknown because all experiments in extracting auxin from root tips by placing them on agar failed (21). Boysen Jensen (8), however, succeeded by adding 10 per cent glucose to the agar. If root tips are put on this glucose-agar for some time the latter will contain g. s.. The explanation for this result is that the tips do not contain enough reserve food materials to produce auxin by themselves but with the help of glucose they can. Lately Thimann (62) could confirm the exosmosis of g. s. from root-tips placed on glucose-agar but he never obtained quite as much auxin from them by this method as by extracting with chloroform. Coleoptile tips, on the other hand, continue to produce g. s. for many hours when placed on agar. His conclusion is that auxin only accumulates in the root tip but is not formed there (in accordance with the polarity theory (76).

So in the case of roots for the first time we find that g. s. acts as an inhibitor of growth. Cholodny (13), however, pointed out that in this case decreased growth in length is accompanied by increase in thickness.

IX

There are other phenomena which depend on auxin. In most cases definite proof has not been furnished that only auxin is the active principle, so they will be mentioned only briefly. However, in the case of bud-inhibition we are sure that it is produced by

auxin, as Kögl's crystalline auxins produced the effect which was attributed to g. s.

It was established, especially by Dostàl (18), that the reason why axillary buds temporarily or forever stay dormant is to be sought in a correlation phenomenon. In his cases inhibition was produced by the leaves. In young bean plants the end bud and young leaves were most active in inhibiting outgrowth of axillary buds, as Snow (50) demonstrated. In this case the correlation effect proved to be complex because a stimulus passing through the xylem as well as one being transported polarly in basal direction only were active. The latter had to be caused by a phyto-hormone as it diffused through a wound gap.

Now Thimann and Skoog (68) were able to replace the inhibiting effect of the end bud by a constant supply of auxin. Axillary buds on the first two nodes of young bean or pea plants never develop unless the end bud is cut off. As soon as this has been done they start to grow. This development, however, is completely checked if in place of the cut end bud, a block of agar with suitable auxin concentration is stuck and changed every six hours to insure a constant supply. Application of an auxin solution is as effective. Whereas the axillary buds of decapitated plants grow out as rapidly as those on similar plants treated with pure agar, they do not develop at all on the decapitated plants treated with a concentration of 1000–5000 units g. s. per cc., nor on normal plants. The concentration of auxin giving a complete inhibition is roughly the same as the amount of auxin produced by the end buds and this fact in connection with the effectiveness of crystalline auxins proves that in the normal plant, outgrowth of axillary buds is inhibited by auxin flowing from the end bud.

Uhrova (71) inhibited growth of axillary buds of *Bryophyllum* with the exudate of leaves containing g. s. Laibach (39) and his students obtained the same effect with pollen g. s. on outgrowth of axillary buds of the cotyledons.

With his method of applying auxin mixed with lanolin a number of other effects of g. s. were noticed by Laibach (39). In all these cases, however, final proof that auxin action is involved has to be given with crystalline auxins. Among these other effects may be mentioned growth of style and ovary in orchid flowers which occurs after pollination. This phenomenon was studied first by

Fitting (19) and ascribed to a pollen hormone present in the pollinia. Now it can be shown to be produced by g. s. of the orchid pollen (40). G. s. also inhibits shedding of the leaf stalk after the leaf blade has been cut off (39, 71). To prevent the leaf stalk from being severed from the stem some g. s. is applied to the apical cut surface of the stalk.

X

The rôle of the growth-hormone in plants is not limited to direct growth phenomena as described in preceding paragraphs. Processes depending on differential growth also are directed by it. Phototropism as well as geotropism are caused mainly by asymmetric distribution of auxin inside the bending organ. The same thesis can be defended with regard to epinastic movements (73). However, this is not the place to discuss these questions (see future article on Phototropism).

Still it might be worth while to point out that in elongating stem-like organs of the higher plants auxin is always connected with the growth process. This does not mean that all growth phenomena are auxin-phenomena; it only says that one definite part of each growth process is controlled by auxin. For this reason each student of plant growth ought to determine to what extent auxins are involved in the process under investigation. If they play a major part as in tropisms, auxin analysis will give a basis for explanation of the process; analysis of the production, transport and action of auxin furnishes a means of differentiating growth phenomenon into a number of factors, each one liable to yield a clue for a causal explanation.

"Bios" and "*B*-Wuchsstoffe," likewise, will not be treated here; they affect growth of yeasts, *i.e.*, increase in number of cells. Functionally they are more related to wound- and lepto-hormones. Their properties are completely different from auxin (among others, the latter is soluble in ether and "bios" is not).

XI

As mentioned before, the best source of auxin is human (and mammalian) urine. This raises the questions whether this g. s. is synthesized in the animal body and whether it has any significance for its metabolism. Both questions have not been definitely an-

swered as yet. We owe, however, a number of interesting facts pertaining to the source of g. s. in the human body to the investigations of Kögl, Haagen Smit and Erxleben (33). It has been found that generally after meals the auxin *a* concentration in urine is increased. This is not correlated with intake of sugars or starch and proteins have but slight effect. However, after eating fat (butter, vegetable oil), excretion of auxin is enormously increased. This is not due to fat metabolism because hydrogenated oils do not have any effect; the hydrogenation has destroyed the auxin of the fat. In most cases auxin seems to be present in esterified form and is not active until it has been saponified.

Auxin content of urine except after meals is very constant and does not change materially upon fasting. Recently Kögl and coworkers (36) published evidence which makes it extremely likely that this "basal auxin" is hetero-auxin, mainly synthesized from tryptophane or allied substance by bacteria in the bowels.

A complete account of the source of all auxin in urine has not been given so that, as mentioned before, the possibility remains that it is synthesized in the human body (34). In connection with this possibility and in view of the considerable amounts of auxin present in all organs of mammals and man the question has been raised whether auxin has physiological significance in the animal organism. The fact in itself that auxin is present in carcinomata in about twice the concentration present in other parts of the body (31, 42) does not have any significance because its concentration in urine is many times higher still. In tissue cultures addition of auxin did not affect growth of fibroblasts. However, as serum, the culture medium, contains auxin this result is not conclusive.

As a general conclusion from the facts known we must say that there is no convincing evidence either in favor or against a synthesis or a physiological rôle of auxin in the animal body. The fact in itself that the presence of g. s. is easily demonstrated in extremely small amounts partially explains its discovery in animal tissues; this technical advantage, however, does not warrant any physiological importance of auxin for the animal.

Other reviews of this field are to be found in citations 2, 11, 22, 41, 51, 64, 74.

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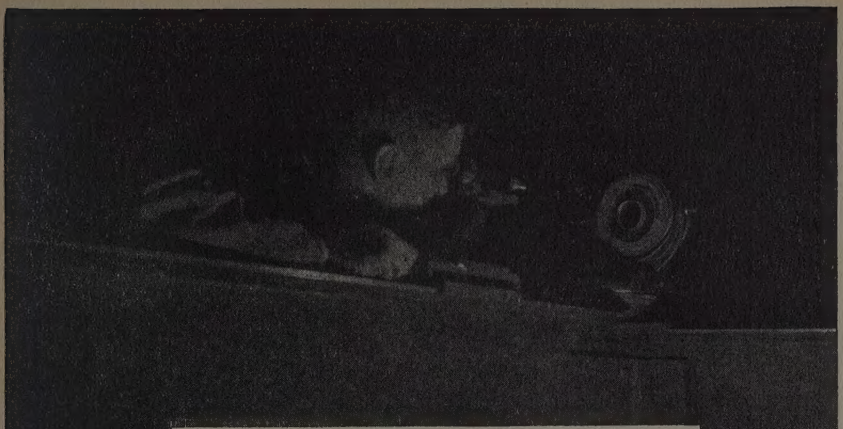

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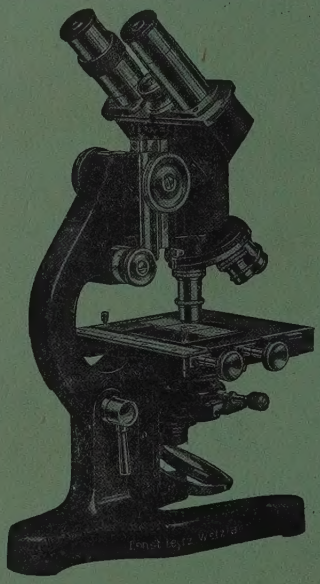

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